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Simian Virus 40-Transformed Cells Express New Species of Proteins Precipitable by Anti-Simian Virus 40 Tumor Serum

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In addition to the virus-coded large-T and small-t antigens, two new classes of proteins were immunoprecipitated by anti-simian virus 40 (SV40) tumor serum from extracts of various SV40-transformed cell lines. These were as follows: (i) proteins (termed "super-T proteins") with an M_r higher than that of large-T antigen (86,000), which were found in many SV40-transformed cell lines derived from mouse and rat cells (super-T proteins and large-T antigen appeared to have closely related structures as judged by the Chromobead elution patterns of their methionine-labeled tryptic peptides); (ii) proteins (termed "55K proteins") with an M_r ranging from 50,000 to 60,000, which were present in all SV40-transformed cell lines examined so far, including those obtained by chromosome-mediated gene transfer. The 55K proteins were not structurally related to large-T antigen, as judged by the Chromobead elution patterns of their methionine-labeled tryptic peptides. Our data are compatible with the assumption that the 55K proteins are largely or totally cell coded.

Immunoprecipitation of cell extracts with anti-simian virus 40 (SV40) tumor serum has led to the identification of the SV40-coded large-T and small-t antigens with molecular weights of approximately 86,000 (86K) and 19,000 (19K), respectively (14, 21, 28-30, 38). The SV40 early mRNA's coding for these antigens have been characterized (1, 7, 22, 26). Both of these early viral proteins may be involved in the establishment or the maintenance of the transformed state (2-4, 7, 9, 11, 20, 25, 31, 36). Furthermore, it has been reported (6, 8, 16, 18, 21, 24, 30) that in many SV40-transformed cell lines, in addition to large-T and small-t antigens, one or several new proteins that react with anti-SV40 tumor serum can be detected.

We have carried out an extensive study of these new species of immunoprecipitable proteins produced in a variety of SV40-transformed cell lines originating from different animal species (human, monkey, mouse, rat, Chinese hamster, Syrian hamster). The results presented in this paper show that these proteins appear to fall into two groups, as follows. (i) Proteins (termed "super-T proteins") with an apparent molecular weight higher than that of large-T antigen are found in some transformed cell lines derived from mouse and rat. In all instances tested they share most or all tryptic peptides with large-T antigen and are mostly or totally virus coded. (ii) Proteins (termed "55K proteins") with an apparent molecular weight in the range 50K to 60K are found in all SV40-transformed cell lines so far examined, including those obtained by chromosome-mediated gene transfer. They do not share common tryptic peptides with large-T. All the data presently available suggest that the 55K proteins may be cell coded and are normally not expressed in adult cell types but are "derepressed" during the transformation by SV40.

MATERIALS AND METHODS

The different SV40-transformed cell lines used in this study are listed in Table 1. Confluent cultures of subcloned epithelioid CV1 monkey cells (19) were infected at a multiplicity of infection of 30 to 50 PFU per cell with wild-type SV40, large-plaque SVLP strain (41). Confluent cultures of primary baby mouse kidney (BMK) cells were prepared and infected with SV40 (SVLP) as previously described (23).

Immunoprecipitation and electrophoresis of proteins extracted from infected and transformed cells. The transformed cell lines were seeded at 5×10^6 cells per 10-cm petri dish (Corning). Forty-eight hours after seeding, the cultures were incubated for 1 h in methionine (or phosphate)-free Eagle minimal essential medium and then labeled for 3 h either with 25 to 50 μCi of L-[^{35}S]methionine per ml (750 to 930 Ci/mmol; The Radiochemical Centre, Amersham, England) or with 100 μCi of $^{32}PO_4$ per ml (C.E.A., France) in methionine (or phosphate)-free medium.

SV40-infected BMK and SV40-infected CV1 cells were labeled 24 h and 72 h, respectively, after infection, with the same radioactive molecules, specific activities, length of time, and media as described for trans-

TABLE 1. *SV40-transformed cell lines used in this study*

Cells	Animal species and tissue	Comments	References
SVMK cl. 9	Mouse (CBA) kidney	Transformed by SV40	43
SVMK cl. 11	Mouse (CBA) kidney	Transformed by SV40	43
V11 F1 cl. 1	Rat (Wag) embryonic lung	Transformed by SV40 DNA form I	L. Daya-Grosjean, C. Lasne, P. Nardeux, and R. Monier, manuscript in preparation
V15 FP1 cl. 1	Rat (Wag) embryonic lung	Transformed by the *Bam*HI-*Hpa*II-generated early fragment of SV40 DNA (map position 0.14–0.73)	
CHK-SVLP	Chinese hamster kidney	Transformed by SV40	17
E1[a]	Syrian hamster	Transformed by SV40	
TSV5 cl. 2	Syrian hamster	Derived from a primary tumor induced by SV40	41
TSV11	Syrian hamster	Derived from a primary tumor induced by SV40	41
EHSVi	Syrian hamster	Transformed by SV40	41
BSC-SV cl. 1	African green monkey kidney	Transformed by SV40	39
WI_{38} VAD	Human skin	Transformed by SV40	12
ME ch. V11 cl. 1-LS[b]	Mouse (Swiss) embryo	Transformed upon exposure to chromosomes isolated from SV40-transformed V11 F1 cl. 1 cells.	
ME ch. V11 cl. 1-A[c]	Mouse (Swiss) embryo		
RE ch. V11 cl. 5-LS[b]	Rat (Wistar) embryo		
RE ch. V11 cl. 6-LS[b]	Rat (Wistar) embryo		

[a] This line (a gift of P. Burtin) was isolated by G. I. Deichman.
[b] Clone isolated in low serum (LS).
[c] Clone isolated in soft agar (A).

formed cells. After labeling the cells were scraped, washed twice with ice-cold phosphate-buffered saline, and suspended at a concentration of 5×10^7 cells per ml in Tris-buffered saline, pH 8 (40) containing 0.5% Nonidet P-40 (Shell Chemical Co.), 10% glycerol, and 2 mM each of diisopropylfluorophosphate (Serva) and sodium *p*-hydroxy mercuribenzoate (Sigma) as protease inhibitors. After 20 min at 4°C, the extract was centrifuged for 30 min at 30,000 × *g*. The supernatant was brought to 0.5 mg/ml in bovine serum albumin and 2 mM in methionine (or 20 mM in phosphate) and then incubated with 2 μl of hamster anti-SV40 tumor or control hamster serum and 20 μl of settled protein A-Sepharose CL 4B (Pharmacia) as described by Schwyzer (35). The suspension was gently agitated overnight at 4°C, then poured into a column in which the Sepharose formed a layer 1 mm or more in thickness. The Sepharose beads were washed three times with 0.2 ml of buffer containing 0.1 M Tris-hydrochloride (pH 8.8), 0.5 M LiCl, and 1% 2-mercaptoethanol. Immune complexes were then eluted from the Sepharose beads with 50 μl of electrophoresis buffer containing 0.08 M Tris-hydrochloride (pH 6.8), 2% sodium dodecyl sulfate (SDS), 5% 2-mercaptoethanol, 15% (wt/vol) glycerol, and 0.001% bromophenol blue and incubated at 100°C for 10 min before analysis by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) as described by Laemmli (15). Gels were either 6.5% or 12% in acrylamide. Electrophoresis was carried out at room temperature at 20 mA for 6 to 8 h. The gels were stained with Coomassie brilliant blue, destained, dried, and exposed to Kodirex X-ray films, usually for 2 to 4 days. The following molecular weight standards were used to calibrate the gels: RNA polymerase (β', 165K, β, 155K, σ, 87K, α, 39K), phosphorylase a (94K), bovine serum albumin (68K), catalase (60K), ovalbumin (43K), aldolase (40K), chymotrypsinogen (25.7K), trypsin inhibitor (21.5K), and β-lactoglobulin (18.4K).

Antisera. In most experiments the anti-SV40 tumor serum used was a pool of sera obtained from Syrian hamsters bearing tumors induced by inoculation of SV40-transformed TSV5 clone 2 (cl. 2) hamster cells. Other anti-tumor sera were also obtained from Syrian hamsters bearing tumors induced by inoculation of SV40-transformed TSV-11 or EHSVi hamster cells. SV40 TCF hamster antiserum was purchased from Flow Laboratories (catalog no. T-840 H). Mouse antiserum was obtained after repeated intraperitoneal injections of cytoplasmic extracts of SV40-infected mouse kidney cells. Before use, all sera were exhaustively absorbed with mixture of lyophilized extracts of EHB tumors (see below) and of lyophilized normal calf serum.

Control serum was a pool of sera obtained from 20 normal adult Syrian hamsters. Another serum was obtained from Syrian hamsters bearing tumors induced by the inoculation of spontaneously transformed EHB hamster cells.

Cell fractionation and isolation of poly(A)-containing cytoplasmic RNA. Twenty-four hours after seeding, cells of 40 10-cm petri dishes of SV40-transformed (V11 F1 cl. 1 or SVMK cl. 9) cells were washed twice with 5 ml each of ice-cold isotonic buffer I (10 mM triethanolamine, pH 8; 25 mM NaCl; 5 mM

$MgCl_2$; 0.25 M sucrose); cells were lysed with 2 ml of cold buffer I containing 1% Nonidet P-40 per 10 petri dishes. The lysate was homogenized with 3 strokes of a tight pestle in a Dounce tissue homogenizer, and nuclear and cytoplasmic fractions were separated by centrifugation at 1,000 × *g* for 5 min at 4°C. Cytoplasmic RNA was extracted by the phenol-chloroform method (33) and then fractionated by oligodeoxythymidylic acid-cellulose chromatography (33) to obtain the polydenylic acid [poly(A)]-containing cytoplasmic RNA [poly(A)+ cytoplasmic RNA].

Translation of gradient-fractionated transformed cell mRNA. Poly(A)+ cytoplasmic RNA samples (50 to 70 μg of RNA) in 200 μl of a buffer containing 10 mM triethanolamine (pH 7.4), 50 mM NaCl, and 1 mM EDTA (used for preparing sucrose gradients) were centrifuged in 16-ml 15 to 30% (wt/vol) sucrose gradients in a Spinco SW27.1 rotor at 23,000 rpm for 20 h at 20°C. Fractions (0.4 ml) were collected from the top of the tubes with an ISCO 640 density gradient fractionator, and absorbance at 254 nm was monitored simultaneously by using an UA-4 absorbance monitor. Each fraction was precipitated with 2 volumes of ethanol after addition of NaCl (to a concentration of 0.15 M) and 4 μg of 7S-4S RNA isolated from control BMK cell cultures. After ethanol precipitation, the RNA from each fraction was pelleted, dissolved in 10 μl of water, and stored at −20°C. The nuclease-treated rabbit reticulocyte lysate system (27) was programmed with the RNA from each fraction (110 μl of reaction mixture containing 30 μCi of L-[^{35}S]methionine [750 to 930 Ci/mmol]). The incubation was for 1 h at 30°C. The reaction was stopped by adding Nonidet P-40 to a final concentration of 2%. The translation products were immunoprecipitated with control and anti-SV40 tumor sera, and analysis was carried out by gel electrophoresis, as described above.

Chromatography of tryptic peptides on chromobeads P. The procedures followed for elution of protein from acrylamide gels, trypsin treatments, and subsequent analysis of peptides by ion-exchange chromatography were essentially those described by Vogt et al. (42). Labeled proteins to be fingerprinted were eluted from the polyacrylamide gel slices by shaking in 0.20 M $(NH_4)_2CO_3$ (pH 8.5), containing 0.1% SDS, at 37°C for 24 h. The supernatant was removed, and the extraction procedure was repeated. The two eluates were pooled. Aliquots were used to check the eluted proteins for their purity. A 100-μg sample of bovine serum albumin was added as a carrier to the eluted proteins (in about 3 ml), and the proteins were precipitated by adding an equal volume of 40% trichloroacetic acid. The mixture was allowed to stand at 4°C for 16 h. After centrifugation at 5,500 × *g* for 30 min at 4°C, the pellet was resuspended in 1 ml of 0.1 M NaOH and then reprecipitated with 0.5 ml of 60% trichloroacetic acid (4°C, 16 h); this step was repeated once more. After the last centrifugation the pellet was oxidized for 1 h at 4°C in 100 μl of performic acid, the oxidation was terminated by the addition of 1 ml of cold distilled water, and the solution was subsequently lyophilized. The lyophilized material was resuspended in 0.5 ml of 50 mM $(NH_4)_2CO_3$ (pH 8.5), and lyophilized again. For tryptic digestion the samples were solubilized in 0.2 ml of 50 mM $(NH_4)_2CO_3$ (pH 8.5) in the presence of 10 μg of trypsin (266 U/mg, L-1-tosylamide-2-phenylethyl chloromethyl ketone-treated; Worthington Biochemical). After 1 h of incubation at 37°C, 10 μg of trypsin was added again, incubation was continued for 1 more hour, and then the peptide solution was lyophilized. The sample was resuspended in 0.5 ml of buffer A (360 ml of water, 1.5 ml of pyridine, 140 ml of acetic acid), clarified by centrifugation, and applied to a column (21 by 0.9 cm) of Chromobeads P (Technicon Chemical, France) which had been equilibrated previously with buffer A. The column was operated at 60°C, and the peptides were eluted with a pyridine-acetate gradient. The gradient ranged from pH 2.4 to pH 4.8 and from 0.04 M to 2 M pyridine in exponentially increasing concentrations and was generated by an automatic gradient maker (Mixograd, Gilson). The flow rate was adjusted to 18 ml/h with a proportioning pump (Technicon). A total of 225 fractions of 3 ml each were collected in glass vials. The fractions were dried in an oven at 100°C, resuspended in 200 μl of 10 mM HCl, and counted in 3 ml of scintillation liquid (Pico-fluor TM 15 scintillator, Packard).

RESULTS

Evidence for the presence in SV40-transformed cell lines of additional proteins immunoprecipitable with anti-SV40 tumor serum. Various SV40-transformed (or SV40-infected) cell cultures were labeled with [^{35}S]methionine or $^{32}PO_4$. After labeling, the proteins were extracted from the cells and immunoprecipitated by anti-SV40 tumor serum; the immunoprecipitates were analyzed on SDS-PAGE. Figure 1 shows the migration patterns of radioactive immunoprecipitable proteins extracted from SVMK cl. 9 (mouse) cells and V11 F1 cl. 1 (rat) cells which were labeled with either [^{35}S]methionine or $^{32}PO_4$. These patterns show the typical occurrence of the band of large-T with an apparent molecular weight (M_r) of 86K, comigrating with the large-T antigen extracted from BMK cells abortively infected with SV40. (In a 6.5% polyacrylamide gel the small-t antigen has too high a mobility to be detectable.) In addition, new bands were found in both [^{35}S]-methionine- and $^{32}PO_4$-labeling experiments, and the corresponding immunoprecipitable proteins fell into two groups: (i) those with an M_r higher than that of large-T and termed super-T proteins, and (ii) those with an M_r ranging between 50K and 60K, operationally termed 55K proteins.

As seen in Fig. 1a, SVMK cl. 9 cells produce several super-T proteins (a doublet at 93K and a single band at 90K) and one 55K protein. The 93K, 90K, and large-T (86K) proteins produced by SVMK cl. 9 cells are encoded by three distinct mRNA's (see below). V11 F1 cl. 1 cells produce

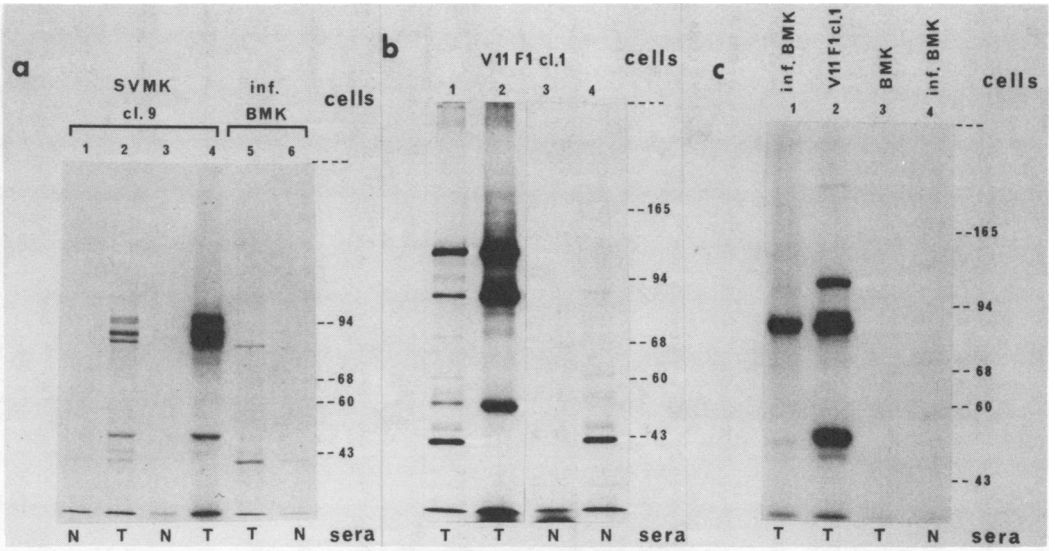

FIG. 1. *SDS-polyacrylamide gel autoradiograms of labeled immunoprecipitated proteins from SV40-transformed SVMK cl. 9 and V11 F1 cl. 1 cells and from SV40-infected BMK cells (inf. BMK). The sera were normal hamster serum (N) and anti-SV40 tumor serum (T) as indicated. The cells were labeled with [^{35}S]methionine (a, tracks 1, 2, 5, and 6; b, tracks 1 and 4) or $^{32}PO_4$ (a, tracks 3 and 4; b, tracks 2 and 3; c). The gels contained 6.5% polyacrylamide.*

a major super-T protein (115K) and one 55K protein in addition to large-T antigen (Figure 1b and c). Super-T, large-T antigen, and 55K protein were not observed in extracts of the corresponding cells precipitated by normal hamster serum. These proteins are phosphorylated, as indicated by their ability to incorporate ^{32}P. Large-T antigen and super-T proteins could be detected by Coomassie blue staining, whereas 55K proteins were obscured by the presence of hamster immunoglobulin G heavy chains in that size range (see below, Fig. 3a).

In the next experiment we compared the $^{32}PO_4$-labeled immunoprecipitable proteins present in SV40-transformed cell lines derived from a variety of animal species (human, monkey, Chinese or Syrian hamster, rat, mouse) (Fig. 2). Immunoprecipitable proteins from cells lytically infected with SV40 were also analyzed in an identical SDS-polyacrylamide gel (Figure 2b and c). It is striking that, whereas super-T proteins were detected only in the SVMK mouse cell line (cl. 9 and cl. 11) and the V11 F1 cl. 1 rat cell line, an immunoprecipitable 55K protein(s) was present in all transformed cell lines tested, including (Fig. 2c) the rat cell line V15 FP1 cl. 1 obtained by transformation with the *Bam*HI-*Hpa*II-generated early fragment of SV40 DNA. The mobility of the 55K protein appears to be slightly different from one animal species to another. Moreover, with the Chinese hamster cell line CHK-SVLP the migration pattern revealed the presence of two weak bands in the range of mobility of 55K proteins (Fig. 2b). The 55K proteins were not observed in extracts of the SV40-transformed cells precipitated by normal hamster serum or in extracts of BMK or CV1 cells not transformed by SV40 (Fig. 1 and 2).

In SV40-infected CV1 monkey cells a faint 58K band was detectable (Fig. 2b and c) when the infection was performed at high multiplicity and on confluent cell monolayers.

A faint 55K band was also detectable in BMK cells abortively infected with SV40 (Fig. 1c).

Identification of immunoprecipitable proteins in SV40-transformed cell lines obtained by chromosome-mediated gene transfer. Primary cell cultures of mouse or rat embryos have been transformed in vitro after a single exposure to metaphase chromosomes from SV40-transformed V11 F1 cl. 1 rat cells (5). Extracts prepared from the permanent cell lines thus transformed (transferent cell lines) were immunoprecipitated with SV40 anti-tumor serum and analyzed by SDS-PAGE. This analysis (Fig. 3) demonstrates that three out of four transferents (ME ch. V11 cl. 1-LS [mouse]; ME ch. V11 cl. 1-A [mouse]; RE ch. V11 cl. 5-LS [rat]) contain a super-T protein with an apparent M_r of 90K, in addition to the typical large-T band. In one transferent (RE ch. V11 cl. 6-LS [rat]) only one immunoprecipitable protein was

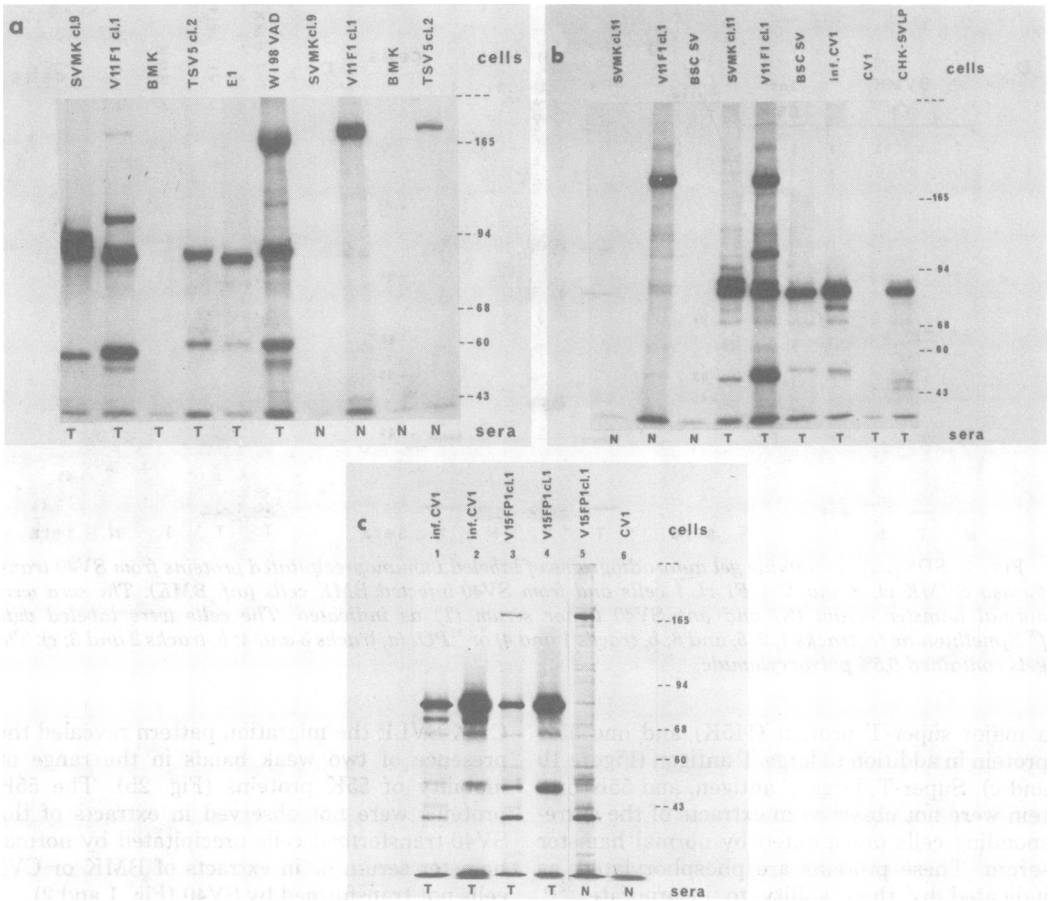

FIG. 2. *SDS-polyacrylamide gel autoradiograms of labeled immunoprecipitated proteins from extracts of SV40-transformed cells and of control CV1 and SV40-infected CV1 cells (inf. CV1). The sera were normal hamster serum (N) and anti-SV40 tumor serum (T) as indicated. The cells were labeled with [^{35}S]methionine (c, tracks 1, 3, and 5) or $^{32}PO_4$ (a, b; c, tracks 2, 4, and 6). The gels contained 6.5% polyacrylamide.*

detectable in the size range of large-T, with an M_r of about 88K. The 115K super-T protein observed in the chromosome donor cell line was not detectable in any transferent cell line tested. The autoradiogram of immunoprecipitable species shown in Fig. 3b also revealed that every transferent line contains a 55K protein. A careful inspection of the mobility patterns suggests that the band of 55K protein is slightly faster in the mouse than in the rat transferent cell lines. A similar difference in the 55K protein mobility is also detectable between the other SV40-transformed mouse and rat cell lines examined (Fig. 2a and b).

Immunoprecipitation of super-T, large-T, and 55K proteins from V11 F1 cl. 1 rat cells by antisera from various origins. In the next experiment we compared the ability of antisera from various origins to immunoprecipitate the super-T, large-T, and 55K proteins. The following sera were used: (i) sera from hamsters bearing tumors derived from injection of one of the three different hamster cell lines TSV5 cl. 2, TSV11, or EHSVi; (ii) the hamster anti-SV40 TCF purchased from Flow Laboratories; (iii) a mouse antiserum raised against extracts from SV40-infected BMK cells; (iv) a serum obtained from Syrian hamsters bearing tumors induced by inoculation of spontaneously transformed EHB hamster cells.

A preparation of ^{32}P-labeled proteins extracted from V11 F1 cl. 1 rat cells was obtained as described in Materials and Methods. Portions of the rat protein preparation were reacted with the various sera. The migration patterns of the immunoprecipitated polypeptides were analyzed in an SDS-polyacrylamide gel, and the ^{32}P-labeled polypeptides were visualized by autoradiography (Fig. 4). When the cell extract was reacted with any of the hamster anti-SV40 tu-

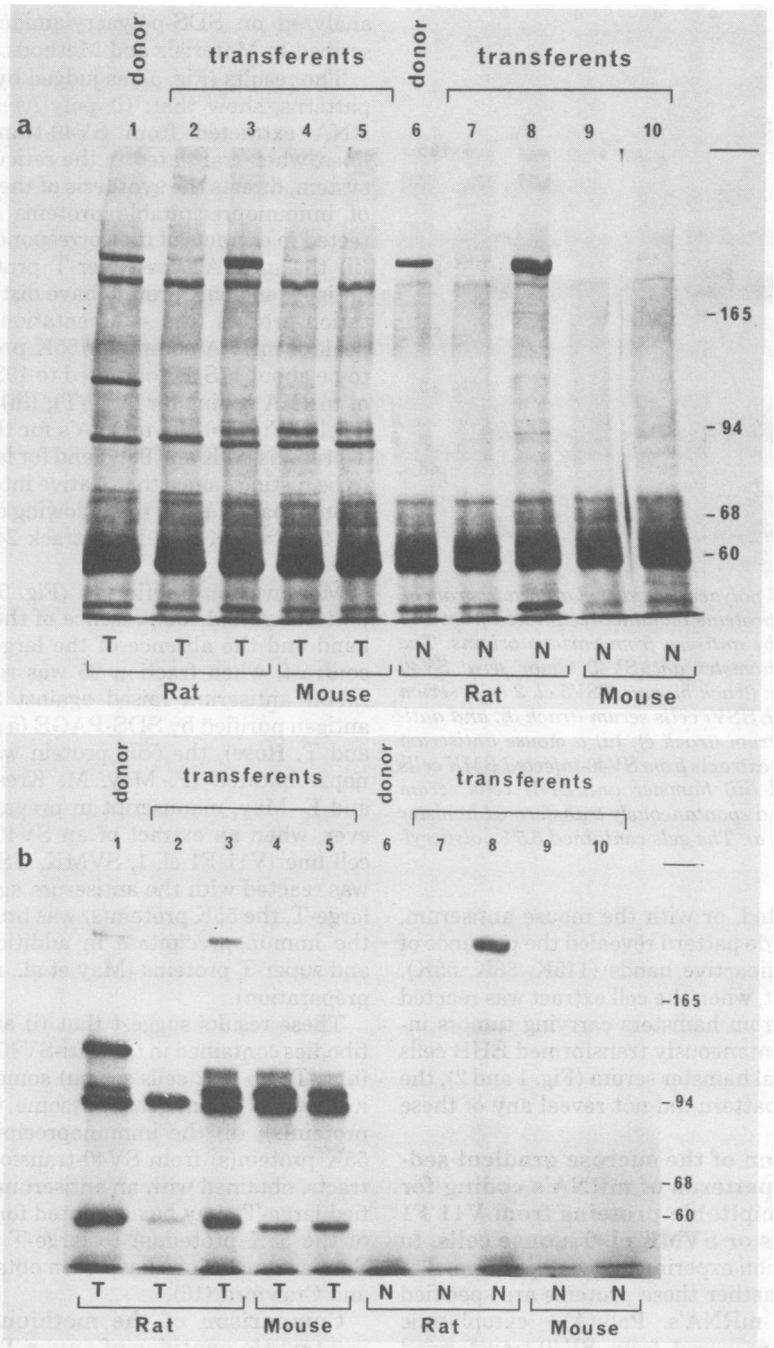

FIG. 3. *SDS-PAGE of $^{32}PO_4$-labeled immunoprecipitated proteins from SV40-transformed cell lines obtained by chromosome-mediated gene transfer. (a) Coomassie blue staining; (b) autoradiogram. The sera were normal hamster serum (N) and anti-SV40 tumor serum (T). The chromosome donor rat cell line was V11 F1 cl. 1 (tracks 1 and 6). The transferents were: (i) rat: RE ch. V11 cl. 6-LS (tracks 2 and 7) and RE ch. V11 cl. 5-LS (tracks 3 and 8); (ii) mouse: ME ch. V11 cl. 1-A (tracks 4 and 9) and ME ch. V11 cl. 1-LS (tracks 5 and 10). The gels contained 6.5% polyacrylamide.*

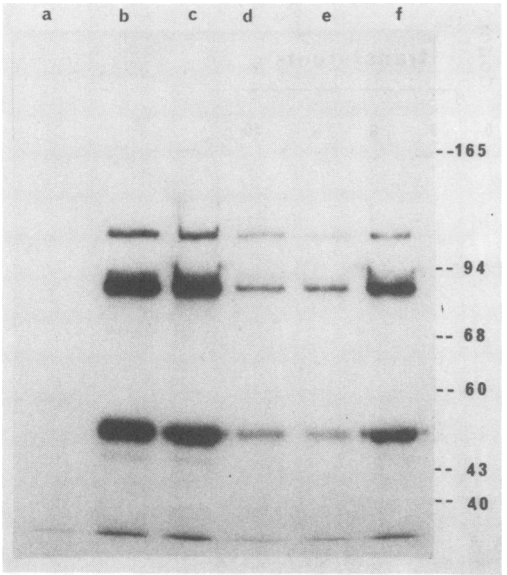

FIG. 4. *SDS-polyacrylamide gel autoradiogram of $^{32}PO_4$-labeled proteins immunoprecipitated from V11 F1 cl. 1 cells by antisera from various origins. The sera were (i) hamster anti-SV40 tumor sera: SV40 TCF antiserum (track b), anti-TSV5 cl. 2 cells serum (track c), anti-EHSVi cells serum (track d), and anti-TSV11 cells serum (track e); (ii) a mouse antiserum raised against extracts from SV40-infected BMK cells (track f); and (iii) hamster anti-EHB cells serum where EHB is a spontaneously transformed hamster cell line (track a). The gels contained 6.5% polyacrylamide.*

mor sera tested, or with the mouse antiserum, the polypeptide pattern revealed the presence of the same radioactive bands (115K, 86K, 55K).

By contrast, when the cell extract was reacted with serum from hamsters carrying tumors induced by spontaneously transformed EHB cells or with normal hamster serum (Fig. 1 and 2), the polypeptide pattern did not reveal any of these bands.

Comparison of the sucrose gradient sedimentation patterns of mRNA's coding for immunoprecipitable proteins from V11 F1 cl. 1 rat cells or SVMK cl. 9 mouse cells. In vitro translation experiments were performed to determine whether these proteins are specified by different mRNA's. Poly(A)+ cytoplasmic RNAs were prepared from SV40-transformed V11 F1 cl. 1 rat cells or SVMK cl. 9 mouse cells. Each RNA preparation was fractionated on an aqueous sucrose gradient. Gradient fractions were collected, precipitated, and translated in the reticulocyte system; the translation products were reacted with control and anti-SV40 tumor serum (hamster anti-TSV5 cl. 2 cells serum) and analyzed on SDS-polyacrylamide gels, as described in Materials and Methods.

The results (Fig. 5), as judged by the mobility patterns, show that: (i) poly(A)+ cytoplasmic RNA extracted from SV40-transformed cell lines, when translated in the reticulocyte lysate system, directs the synthesis of the same species of immunoprecipitable proteins as those detected in extracts of the corresponding cell lines; (ii) the mRNA's for super-T proteins, large-T antigen, and 55K protein have distinct sedimentation profiles (the sedimentation value of the peak of mRNA coding for 55K protein appears to be about 17S as compared to 19S for the peak of mRNA coding for large-T); (iii) in SVMK cl. 9 cells (Fig. 5b) the mRNA's for the two super-T proteins (93K and 90K) and for large-T appear to be distinct, since the relative intensities of the protein bands are in the following order: in track 21, 93K > 90K > 86K; in track 24, 86K ~ 90K > 93K.

Moreover, in fraction 25 (Fig. 5) the peptide patterns reveal the presence of the 55K protein band and the absence of the large-T band. By contrast, when fraction 25 was reacted with a rabbit antiserum raised against SV40 large-T antigen purified by SDS-PAGE (a gift of R. Weil and T. Rose), the 55K protein was not immunoprecipitated (P. May, M. Kress, M. Lange, and E. May, manuscript in preparation). However, when an extract of an SV40-transformed cell line (V11 F1 cl. 1, SVMK, TSV5 cl. 2, etc.) was reacted with the antiserum against purified large-T, the 55K protein(s) was brought down in the immunoprecipitate in addition to large-T and super-T proteins (May et al., manuscript in preparation).

These results suggest that (i) among the antibodies contained in the anti-SV40 tumor serum (anti-TSV5 cl. 2 cells serum) some react specifically with T antigen and some with the 55K protein(s); (ii) the immunoprecipitation of the 55K protein(s) from SV40-transformed cell extracts, obtained with an antiserum against purified large-T, may be accounted for by a binding of the 55K protein(s) to large-T (or super-T). Similar conclusions have been obtained by Lane and Crawford (16).

Comparison of the methionine-containing tryptic peptides of super-T or 55K proteins from V11 F1 cl. 1 rat cells with those of large-T antigen. We compared the methionine-containing tryptic peptides of super-T or 55K protein from V11 F1 cl. 1 rat cells with those of large-T antigen to determine whether there was a structural relationship between these proteins. Super-T and 55K proteins were

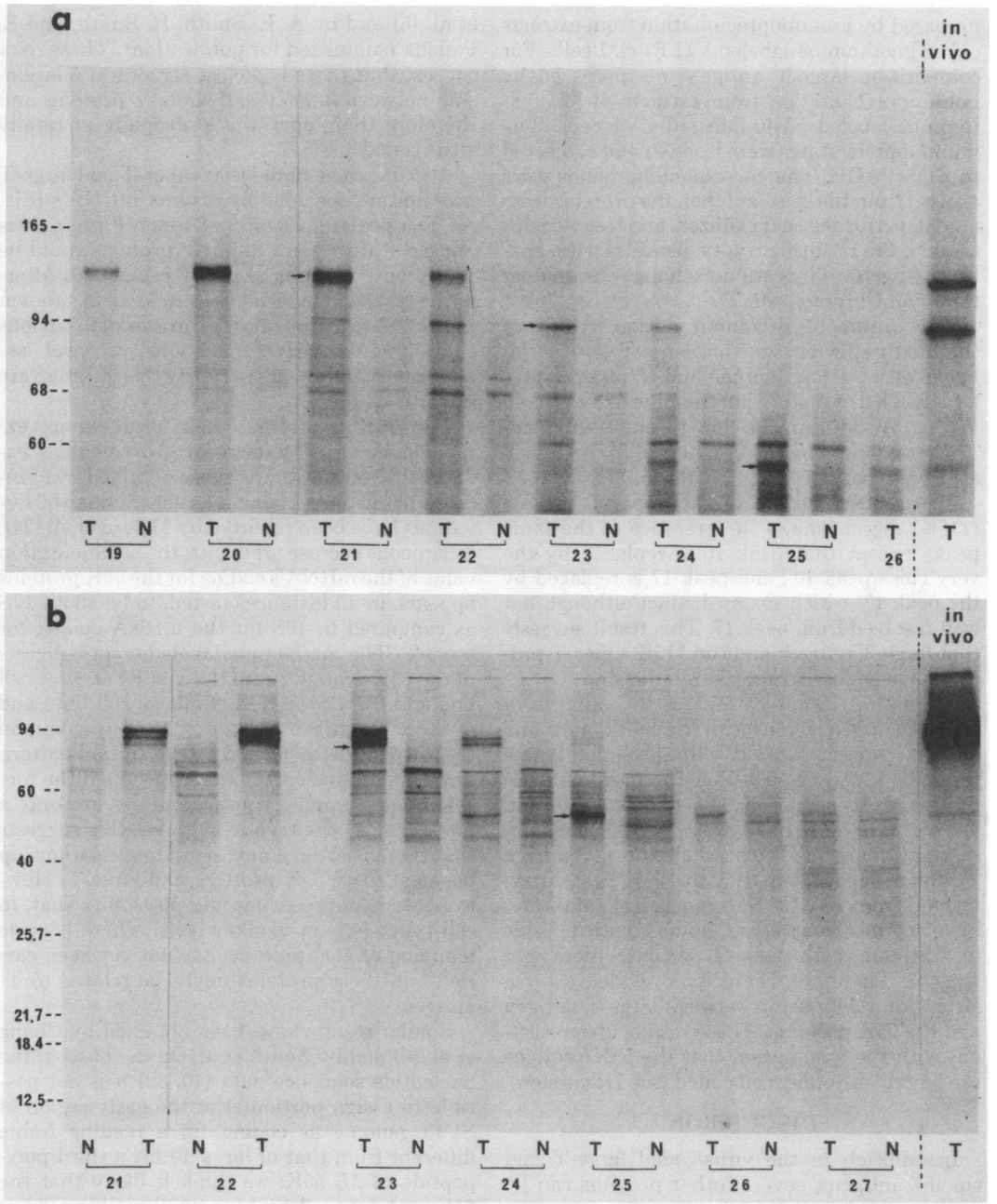

FIG. 5. *Sedimentation profiles of mRNA's coding for super-T, large-T, and 55K proteins from V11 F1 cl. 1 and SVMK cl. 9 cells. Poly(A) + cytoplasmic RNA from V11 F1 cl. 1 (a) or SVMK cl. 9 (b) was fractionated on a 16-ml linear 15 to 30% (wt/vol) sucrose gradient by centrifugation in a Spinco SW 27.1 rotor at 23,000 rpm for 20 h at 20°C. Forty 0.4-ml fractions were collected. The RNA from each fraction was ethanol precipitated in the presence of 4 μg of carrier RNA and redissolved in 25 μl of water. The reticulocyte lysate system was programmed with 5 μl of RNA from each fraction, and the translation products were immunoprecipitated with anti-SV40 tumor serum (T) (and with normal hamster serum [N] for comparison) and analyzed by SDS-PAGE. Sedimentation was from right to left. Fraction 24 corresponds to the position of 18S rRNA sedimented in a parallel gradient. The gels contained 6.5% (a) or 12% (b) polyacrylamide. The figures in the lowest line indicate the fraction numbers. The figures on the left indicate the positions of the molecular weight markers (apparent $M_r \times 10^3$). Control, track at the far right, was $^{32}PO_4$-labeled immunoprecipitated proteins from extracts of V11 F1 cl. 1 cells (a) or of SVMK cl. 9 cells (b).*

prepared by immunoprecipitation from extracts of [^{35}S]methionine-labeled V11 F1 cl. 1 cells. For comparison, large-T antigen was prepared by immunoprecipitation from extracts of [^{3}H]methionine-labeled SV40-infected CV1 cells. The immunoprecipitates were isolated and subjected to SDS-PAGE. The corresponding bands were excised from the gels, and then the proteins were eluted, performic acid oxidized, and treated with trypsin; the resulting tryptic peptides were analyzed (together) by cation-exchange chromatography on Chromobeads P.

The Chromobead column elution pattern of the mixture of digests from large-T antigen labeled with [^{3}H]methionine and of super-T protein labeled with [^{35}S]methionine is shown in Fig. 6a. Aside from the flow-through, there are 17 characteristic peaks of material from large-T antigen, labeled 1, 2, 3, . . . , 15, 16, 17.

The elution profile of the super-T protein (115K) digests shows the presence of the same peaks except that peak 10 is replaced by the very close peak 10′, and peak 17 is replaced by the peak 17′ which seems distinct although not well resolved from peak 17. This result suggests that large-T antigen and the 115K super-T protein have a close structural relationship.

From the elution pattern of the mixture of large-T antigen labeled with [^{3}H]methionine and of 55K protein labeled with [^{35}S]methionine (Fig. 6b) it can be seen that the profile of 55K protein shows six peaks, labeled a, b, c, d, e, and f, which are all distinct from the 17 peaks observed for large-T. We have also observed from a similar experiment that the 55K protein extracted from SVMK cl. 9 mouse cells does not have any methionine-containing tryptic peptide in common with large-T antigen (data not shown). Therefore, there is no evidence for a structural relationship between large-T antigen and the 55K proteins. These results are consistent with the assumption that the 55K proteins are largely or totally cell coded (see Discussion).

DISCUSSION

In addition to the virus-coded large-T and small-t antigens, several other proteins can be immunoprecipitated from extracts of various SV40-transformed cell lines by using anti-SV40 tumor serum. We classify these proteins operationally into two categories, as follows.

The super-T proteins. These proteins have an M_r higher than that of large-T. They were found in many SV40-transformed cell lines of mouse and rat origin. The tryptic digests of super-T and of large-T proteins showed similar elution patterns on a Chromobead column. Analogous results have been obtained by Chang et al. (6) and by A. E. Smith, R. Smith, and E. Paucha (submitted for publication). These data suggest that there is a close structural relationship between large-T and super-T proteins and therefore that super-T is principally or totally virus coded.

Also, it can be noted that super-T and large-T proteins are specified by distinct mRNA's (Fig. 5). The genetic origin(s) of super-T proteins is not clear at present. Super-T proteins could be partly virus coded and partly cell coded. Alternatively, they could be the products of tandem insertions of the integrated virus or of insertions containing partial duplications of viral sequences. Experiments to clarify this question are under way.

The 55K proteins. These proteins are expressed in all SV40-transformed cell lines examined so far, including those obtained by chromosome-mediated gene transfer. Similar observations have been reported by Melero et al. (24). In aqueous sucrose gradients, the sedimentation value of the mRNA's coding for the 55K proteins appears, in all instances tested, to be about 17S as compared to 19S for the mRNA coding for large-T. The methionine-labeled tryptic digests of the 55K protein (from the V11 F1 cl. 1 rat cell line and from SVMK cl. 9 mouse cell line) and of large-T antigen did not share any common tryptic peptide, as judged by the elution pattern using a Chromobead column. Although the methionine-containing tryptic peptides are only a portion of all the tryptic peptides, this suggests that there is little, if any, structural relationship between these 55K proteins and large-T. However, we cannot exclude the possibility that, in cell types (e.g. in monkey cells) where peptide mapping of 55K proteins has not yet been carried out, these proteins might be related to T-antigen.

Similar results have been obtained by Chang et al. (6) and by Smith et al. On the basis of the nucleotide sequence data (10, 32) it is not possible to assign portion(s) of the early region of SV40 genome as coding (in a reading frame different from that of large-T) for a third polypeptide of M_r 55K; we think it likely that the 55K proteins are largely or totally cell coded but that they are not expressed in adult cell types. It is noteworthy that S. Segal, A. J. Levine, and G. Khoury (Cell, in press) observed the presence in both SV40-infected and uninfected mouse teratocarcinoma cells (F9) of two distinct polypeptides with apparent M_r's of 105K and 55K, which are immunoprecipitable with anti-SV40 tumor serum.

These results are most easily rationalized with our observation that the electrophoretic mobil-

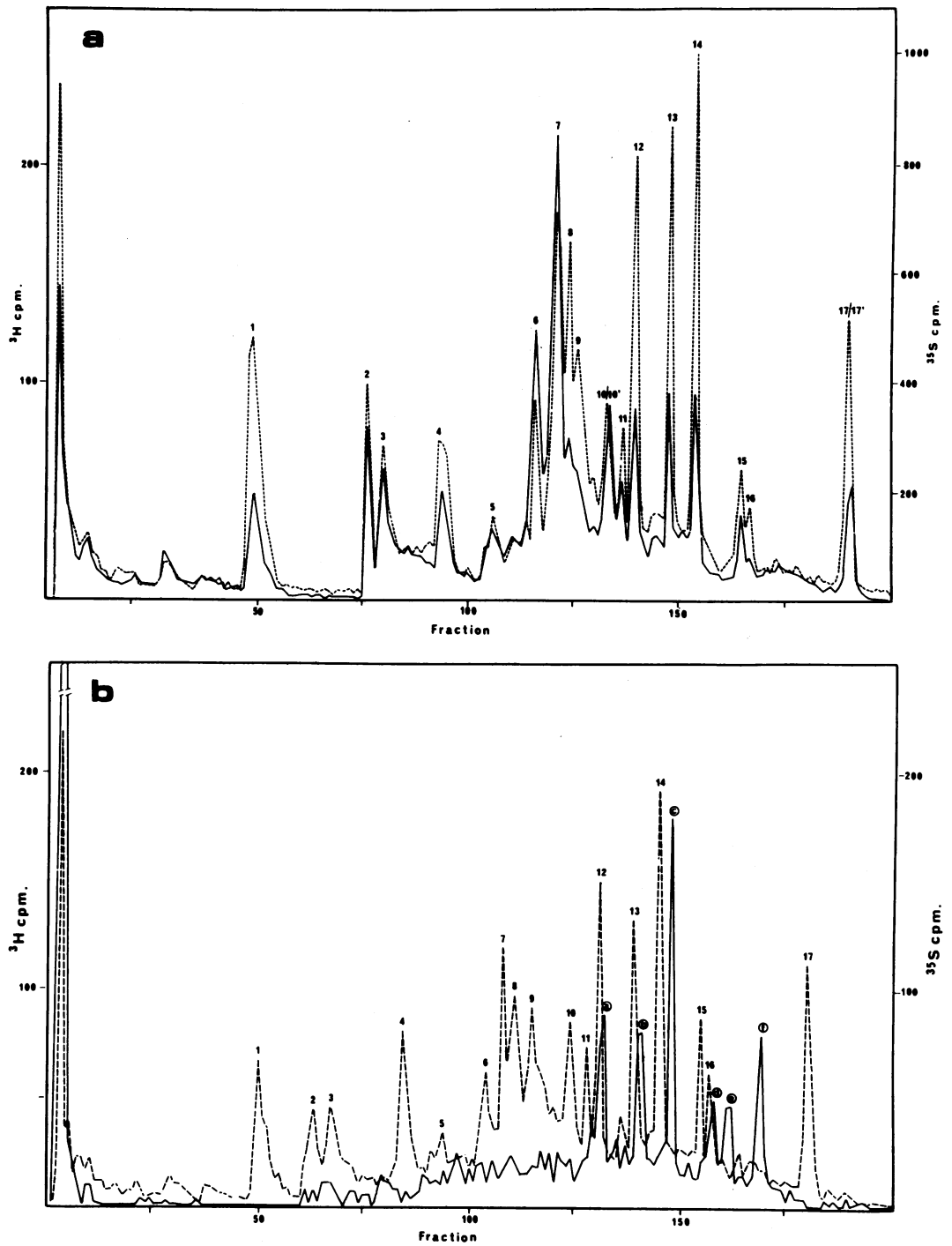

FIG. 6. *Comparison of the methionine-labeled tryptic peptides of super-T or 55K protein from V11 F1 cl. 1 rat cells with those of large-T antigen. [^{3}H]methionine-labeled large-T antigen was prepared by immunoprecipitation from extracts of SV40-infected CV1 cells. The CV1 cell cultures were infected at a multiplicity of 50 to 60 PFU per cell before the cells became confluent (19); they were labeled at 24 to 28 h postinfection with 100 μCi of L-[methyl-^{3}H]methionine per ml (New England Nuclear Corp.; 80 Ci/mmol). Super-T and 55K proteins were prepared by immunoprecipitation from extracts of [^{35}S]methionine-labeled V11 F1 cl. 1 cells. The bands corresponding to these proteins were excised from preparative gels. The proteins were eluted from the gels and treated with trypsin. [^{35}S]methionine-labeled super-T (a) or 55K protein (b) and large-T antigen were analyzed together by ion-exchange chromatography on P-type Chromobeads. The peptides were eluted with a pyridine-acetate gradient at 60°C. The gradient ranged from pH 2.4 to pH 4.8 and from 0.04 M to 2 M pyridine in exponentially increasing concentration. A total of 225 fractions of 3 ml each were collected at a pumping rate of 18 ml/h. The fractions were evaporated to dryness and counted for ^{3}H and ^{35}S radioactivities. (-----) ^{3}H cpm (large-T); (——) ^{35}S cpm (a, super-T; b, 55K protein).*

ity of the 55K protein(s) varies from one animal species to another, an observation consistent with the fact that the tryptic peptide elution patterns of the 55K proteins vary from one animal species to another, as observed by Chang et al. (6) and by Smith et al. In addition, Edwards et al. (8) have suggested the existence of a species difference in the expression of the 55K protein(s).

Although SV40 and polyoma viruses have a similar genetic organization, the 55K proteins expressed in SV40-transformed cells are not the counterpart of the polyoma middle-T antigen (M_r 55K to 60K) since the latter is polyoma virus coded (13, 34, 37). Rather, the SV40 55K proteins can be compared to the "55K T-antigen" observed in polyoma-infected cells by Hutchinson et al. (13), which is unrelated to the polyoma large-, middle-, or small-T proteins, as determined by tryptic peptide analysis.

In conclusion, it is tempting to assume that the 55K proteins identified in the present study are fetal antigens which are normally not expressed in adult cell types, but that they are derepressed in the course of the virus-induced transformation process. If so, it might be possible to detect similar proteins in cell lines transformed by viruses other than SV40. Indeed, we have recently found that extracts from the polyoma-transformed rat cell line Py BN and from the adenovirus type 5-transformed hamster cell line HT 14-B contain a 55K protein immunoprecipitable by anti-SV40 tumor sera (May et al., manuscript in preparation). The presence of cross-reacting proteins of the same size in cells transformed by other viruses, as well as the nature of the 55K proteins, is under investigation.

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